An *In vitro* Comparative Study of Apical Extrusion of Bacteria Using Hand and Rotary Systems

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Abstract

Background and Objectives: The objective of the study was to compare *Enterococcus faecalis* bacteria extruded apically from extracted teeth after endodontic instrumentation using the hand instrumentation techniques, rotary ProTaper next, and rotary One Shape technique. **Methodology:** A total of 45 freshly extracted human mandibular premolars were used and test apparatus to determine apical extrusion of bacteria in tooth was forced through the rubber stopper of a vial after endodontic access cavity preparation. Two 23-gauge needle were inserted into the vial through the rubber stopper to equalize the air pressure. Entire apparatus was sterilized in an autoclave. A pure culture of *E. faecalis* (MTCC) was used to contaminate the root canal. During incubation, canals were hand instrumented with #10 K-file to carry the bacteria down the length of the canal. The contaminated root canal was dried at 37°C for 24 h. Samples were equally divided into three groups for instrumentation with different techniques. Colonies of bacteria were counted using a colony counter following a classical bacterial counting technique as described by Collins *et al*. The results were given as number of CFU/ml. **Results:** Data regarding the number of bacteria extruded were presented and statistically analyzed. The results indicated that all instrumentation techniques tested caused a measurable apical extrusion of bacteria. The hand instrumentation techniques (crown down) and rotary multiple file techniques extruded more number of bacteria compared to rotary single file (One Shape) system. The manual crown-down technique showed greatest extrusion of bacteria and there was a statistically significant difference ($P < 0.05$) found between the crown-down technique and other rotary multiple and single file Ni–Ti instrumentation techniques. The single file rotary (One Shape) extruded significantly smaller number of bacteria. The crown-down hand instrument technique extruded highest number of bacteria and among the rotary multiple file systems and rotary single file system (One Shape) extruded least number of bacteria. **Conclusion:** Overall, the engine-driven techniques were associated with the least apical extrusion of irrigant and debris (bacteria) hand instrumentation techniques (crown down) and rotary multiple file system extruded large number of bacteria than engine-driven techniques (rotary single file system One Shape).

Keywords: *Enterococcus faecalis*, Extrusion, Hand instruments, Rotary, Single and multiple Ni–Ti files

Introduction

Proper cleansing of the root canal space is considered essential for success in endodontics.[1] The canal is cleaned by eliminating both the organic contents and the contaminated intracanal dentin wall. This goal is accomplished using a thorough chemomechanical root canal preparation composed of mechanical debridement combined with biocidal irrigation.[1] This task is complicated by the fact that root canal anatomy is unpredictable and to clean and shape these canal successfully, it requires appropriately designed cleaning instruments and intracanal irrigation to thoroughly remove the debris or bacteria.[1] The prevention of pain during endodontic treatment is of prime consideration to both clinician and patient.[2] Adequate local anesthetics eliminate most of the pain during treatment, but considerable discomfort is occasionally experienced after endodontic instrumentation due to overextension of debris (infected or otherwise), instruments, paper points, medicaments, or filling materials. During the biomechanical instrumentation of the root canal, dentin chips, pulp tissue fragments, necrotic tissue, microorganisms, and intracanal irrigants may be extruded from the apical foramen into the apical tissues.[3] An instrumentation technique that minimizes apical extrusion of debris or bacteria would be advantageous to both the clinician and the patient. This is of concern since the material extruded from the apical foramen

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may be related to post instrumentation pain or flare-up, which may lead to liquefaction necrosis.[3]

Many factors affect the amount of extruded debris or bacteria such as the improper use of irrigating solutions, long canals, instrumentation methods, canal diameter, larger files (size 50 and above), and type of file used.[2] Irrigation or chemical debridement is accepted as being a necessary aid in the chemomechanical cleansing of root canals. Irrigation assists in debris or bacteria removal. More debris is removed when greater quantities of irrigating solutions are used.[4] Furthermore, the proximity of the irrigation needle to the apex plays an important role in removing root canal debris.[4] The effectiveness of irrigation is questioned in narrow canals, with smaller needles being suggested for better exchange of solution and cleaning.[5] Ram (1977) found that bacteria removal is a function of canal diameter and that effective irrigation may not occur consistently unless canals are enlarged to at least a #40 instrument.[5] However, one study concluded that narrow canals prepared to a minimum diameter (#25) can be effectively flushed of debris if they are prepared with sufficient taper to allow a small needle to be placed in the apical third of the canal.[5] Several studies have indicated that better irrigation is possible with flaring of the cervical portion of the root canal. This allows the irrigants easier access to the apical region and thus easier removal of debris.[5] The length of the canal seemed to strongly influence the amount of extruded material. The amount of material extruded increased in proportion to the length of the canal. Various instrumentation techniques have been advocated to minimize the extrusion of debris apically. Earlier the conventional hand filing with K-files was employed, which extruded considerable amount of debris. This was mainly due to its push-pull motion, which created greater apical pressure and causing a plunger action forcing the debris ahead of the file through the patent apical foramen and out into the periradicular tissue. Ingle has referred to this as the “worm” of necrotic debris.[6] Root canal instrumentation using filing movements may force material to the periapex since, as pointed out by Grossman (1956), files act as pistons.[7] Crown-down preparation technique results in greater extrusion of debris apically, which can cause greater damage to the periapical tissues with subsequent post-operative discomfort to the patient. Omari and Dummer verified that techniques involving linear filing motion such as the crown-down techniques create a greater mass of debris than those involving some sort of rotational action.[8] It acts as a plunger to force the debris ahead of the file through the patent apical foramen and out into the periradicular tissue. According to Roane *et al.,* On the other rotary technique by Pro Taper next,resulted in less number of bacteria apically. Instrumentation is divided into placement, cutting, and removal using only rotary motion on the files.[9] The rotational motion utilized by this technique, design of rotary ProTaper next file, or combinations of both were responsible for less apical extrusion. They tend to pull dentinal debris into the flutes of the file and direct it toward the coronal aspect of the canal.[9] Recently, many advances have been taking place in the preparation techniques and instruments employed. The crown-down or cervical flaring type preparation results in less apical extrusion of debris by reducing the amount of dentin available to push apically and creating a space large enough for debris to be rinsed away in a coronal direction.[5] The rotary instrumentation utilizes the crown-down technique and hence results in less extrusion of debris apically. Debris or bacteria extrusion is a problem with virtually all instrumentation techniques.[5]

Many new techniques are still being developed to reduce the amount of debris or bacteria extruded apically. The purpose of the study was to compare *in vitro* the amount of debris or bacteria extruded apically from extracted teeth using rotary technique, hand instrumentation techniques with single file rotary techniques utilizing nickel–titanium instruments.[6]

Methodology

This study was done in the Department of Conservative Dentistry and Endodontics, Daswani Dental College and Research Centre, Kota, Rajasthan, India, and carried out in the Department of Pathology and Microbiology, Government Medical College, Kota, Rajasthan, India.

Forty-five extracted single-rooted mandibular premolar teeth for the study with unhealthy periodontal conditions and therapeutic extraction for orthodontic purposes were collected from patients referred to the Department of Oral and Maxillofacial surgery, Daswani Dental College and Research Centre, Kota, Rajasthan, India. The teeth were stored in distilled water after extraction after removing tissue debris using a gauge piece.

The extracted teeth were cleaned of any residual tissue. Debridement of all teeth was carried with hand scaling instruments and stored in distilled water at 37°C and the OSHA guidelines were followed for further management and storage of teeth (Annexure I) which states the OSHA and CDC guidelines highlighting the following key criteria for storage of extracted teeth: The teeth are placed in a container which prevents leakage during collection, handling, processing, storage, or shipment. The container must be labeled with the biohazard symbol along with the word “Biohazard” in the required contrast colors or color coded and closed before being stored, transported, or shipped. The teeth should be cleansed of visible blood and gross debris and maintained in hydrated state.

Test apparatus to determine apical extrusion of intracanal bacteria in tooth was forced through the rubber stopper of a vial after endodontic access cavity preparation. Two 23-gauge needle were inserted into the vial through the rubber stopper to equalize the air pressure. Entire apparatus was sterilized in an autoclave. The hole was created in nail varnish that covered the apical foramen using 10 K-file. The tooth with rubber stopper needle unit was placed into the mouth of the vial. The same procedure was repeated to all experimental teeth.

A pure culture of *Enterococcus faecalis* (MTCC) was used to contaminate the root canal. Suspension was prepared by adding pure culture of *E. faecalis* grown in brain–heart infusion broth for 24 h to fresh brain–heart infusion broth. During incubation, canals were hand instrumented with #10 K-file to carry the bacteria down the length of the canal. The contaminated root canal was dried at 37°C for 24 h. Samples were equally divided into three groups for instrumentation with different techniques. Subsequently, after root canal preparation, 100 microliter of saline was taken from experimental vial with use of microtiter pipette, to count the bacteria and incubated in brain–heart infusion agar at 37°C for 24 h. Colonies of bacteria were counted using a colony counter following a classical bacterial counting technique as described by Collins *et al*. The results were given as number of CFU/ml.[8]

Armamentarium

I. Diamond disk with mandrel (no. 12129218, DFS, Germany)
II. Micromotor handpiece (NSK, Japan)
III. Micromotor control box with engine (Dents Ark, India)
IV. Endomotor (Canal Pro CL 2, Coltene, USA)
V. 21 mm No. 10 Hand K-Files (Mani Inc., Tochigi, Japan)
VI. 21 mm No. 15-40 K Files (Mani Inc., Tochigi, Japan)
VII. Endoblock (Dentsply)
VIII. Vernier Caliper (Liberal Traders, India)
IX. One Shape rotary files (Micro-Mega, Besancon, Cedex)
X. ProTaper next rotary files X1, X2, X3, X4, and X5 (Dentsply Maillefer, Switzerland)
XI. Marker pen (Faber castle)
XII. Nail varnish
XIII. 23-gauge needle
XIV. Autoclave
XV. Round bur
XVI. Class laminar air flow cabinet.

Material required

I. Distilled water
II. 0.2% saline (Infutec Healthcare, Hyderabad)
III. EDTA gel (RC Help, Prime Dental)
IV. 10 ml syringes (Dispovan)
V. Brain–heart infusion agar
VI. *E. faecalis*.

Selection of teeth

Inclusion criteria

The following criteria were included in the study:

- Teeth with single, straight roots (curvature less than or equal to 10%)
- Single canals teeth
- Non-carious teeth
- Teeth with completely formed apex.

Exclusion criteria

The following criteria were excluded from the study:

- Fractured teeth
- Teeth with root resorption
- Teeth with severe anatomic variations
- Teeth with calcified canals.

Photographs

Study sample

Armamentarium

Arma

Class 1 laminar air flow

Test apparatus

Statistical analysis

Results are presented in mean±SD. One-way analysis of variance (ANOVA) was used to compare CFU among the groups. Dunnett's D *post hoc* comparison test was used for pairwise comparisons. $P < 0.05$ was considered statistically significant. All the analyses were carried out on SPSS 16.0 version *(Chicago, Inc., USA).

Formula

Mean and standard deviation (SD)

The *sample mean* is the average and is computed as the sum of all the observed outcomes from the sample divided by the total number of events. We use x as the symbol for the sample mean. In math terms,

$$\bar{x} = \frac{1}{n}\sum_{i=1}^{n} x$$

where, n is the sample size and the x corresponds to the observed valued.

We define the *variance* to be

$$s^2 = \frac{1}{n-1}\sum_{i=1}^{n}(x-\bar{x})^2$$

and the *standard deviation* to be

$$s = \sqrt{\frac{1}{n-1}\sum_{i=1}^{n}(x-\bar{x})^2}$$

Dunnett's D

Dunnett's test (also called Dunnett's method or Dunnett's multiple comparison) compares means from several experimental groups against a control group mean to see is there is a difference. When an ANOVA test has significant findings, it does not report which pairs of means are different. Dunnett's can be used after the ANOVA has been run to identify the pairs with significant differences.

As Dunnett's compares two groups, it acts similarly to a t-test. The following formula gives a value that you can use to compare mean differences. The formula is:

$$D_{Dunnett} = t_{Dunnett}\sqrt{\frac{2MS_{S/A}}{n}}$$

Results and Observations

A total of 15 premolars in each group were included in the study and the results were analyzed when we compare the comparison of CFU among the groups. The analysis of variance showed that there was a significant ($P = 0.0001$) difference in CFU among the three groups that are Group 1, Group 2, and Group 3.

The comparison of number of CFU/ml between Group 1 and Group 2. The *post hoc* tests revealed that CFU was significantly ($P = 0.0001$) higher in Group 1 (315.13 ± 63.97) compared to Group 2 (151.87 ± 32.58) (Figure 1 and Table 1).

The comparison of number of CFU/ml between Group 1 and Group 3. The *post hoc* tests revealed that CFU was significantly ($P = 0.0001$) higher in Group 1 (315.13 ± 63.97) compared to Group 3 (28.40 ± 12.50) (Figure 2).

The comparison of number of CFU/ml between Group 2 and Group 3. The *post hoc* tests revealed that CFU was significantly ($P = 0.0001$) higher in Group 2 (151.87 ± 32.58) compared to Group 3 (28.40 ± 12.50).

Table 2 and Figure 3 show the comparison of CFU between Group 2 and Group 3. The *post hoc* tests revealed that CFU was significantly ($P = 0.0001$) higher in Group 2 (315.13 ± 63.97) compared to Group 3 (151.87 ± 32.58).

Table 3 and Figure 2 show the comparison of CFU between Group 1 and Group 3. The *post hoc* tests revealed that CFU was significantly ($P = 0.0001$) higher in Group 1 (315.13 ± 63.97) compared to Group 3 (28.40 ± 12.50).

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Results

Data regarding the number of bacteria extruded were presented and statistically analyzed. The results indicated that all instrumentation techniques tested caused a measurable apical extrusion of bacteria. The hand instrumentation techniques (crown down) and rotary multiple file techniques extruded more number of bacteria compared to rotary single file (One Shape) system.

The manual crown-down technique showed greatest extrusion of bacteria and there was a statistically significant difference ($P < 0.05$) found between the crown-down technique and other rotary multiple and single file Ni–Ti instrumentation techniques. The single file rotary (One Shape) extruded significantly smaller number of bacteria (Table 4 and Figure 4).

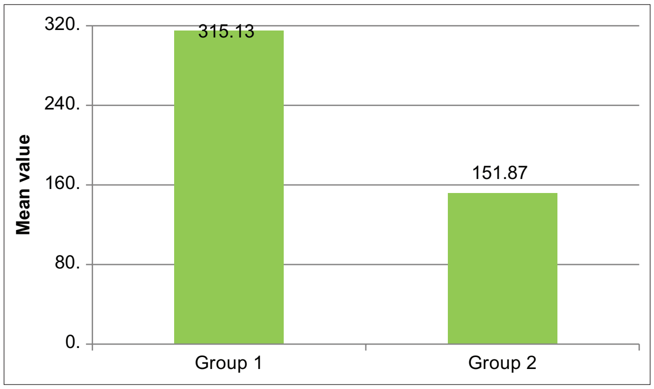

Figure 1: Comparison of number of CFU/ml among Group1 and Group 2

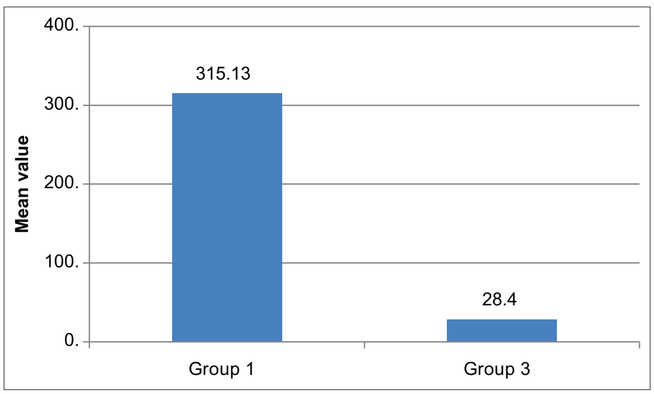

Figure 2: Comparison of CFU between Group 1 and Group 3

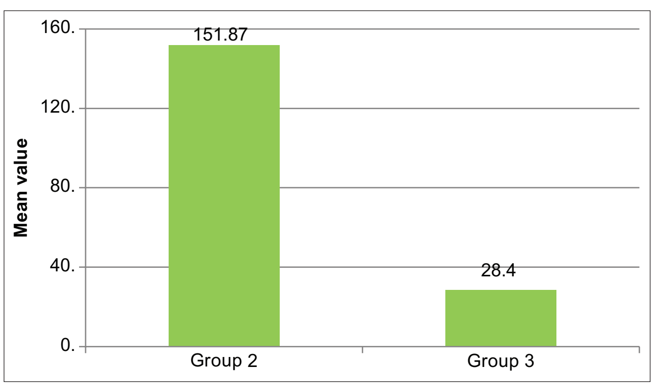

Figure 3: Comparison of CFU between Group 2 and Group 3

Discussion

Proper cleansing of the root canal space is considered essential for success in endodontics.[1] Dentin chips, pulp tissue fragments, necrotic tissue, microorganisms, and intracanal irrigants may be extruded from the apical foramen during canal instrumentation which may lead to periapical inflammation causing post-operative pain or flare-up. In any event, root canal instrumentation should be performed in a manner that minimizes the amount of material extruded into periapical tissue.[3]

In the present study, assessment of extrusion of intracanal bacteria as a result of canal shaping by different instrumentation techniques was carried out. *E. faecalis* was chosen as a bacteriological marker because it can survive alone without symbiotic support from other bacteria. Large amount of bacteria extruded apically in crown-down hand filling technique was due to watch winding and in and out filling motion that acted as piston extruding more amount of bacteria compared to other instrumentation techniques.[4]

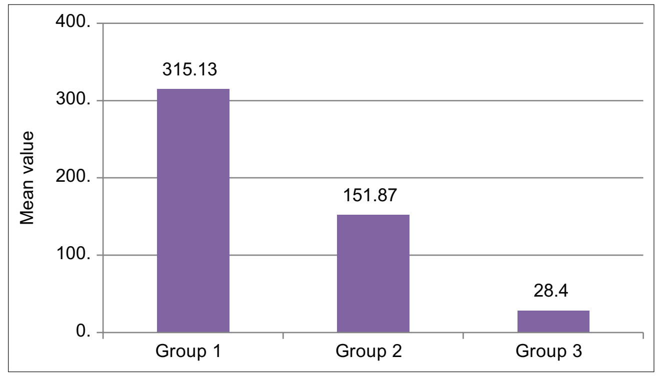

Figure 4: Comparison of number of CFU/ml between Group 1, Group 2, and Group 3

Table 1: Comparison of number of CFU/ml among Group 1 and Group 2

Groups	CFU/ml (Mean±SD)
Group 1	315.13±63.97
Group 2	151.87±32.58
P-value[1]	0.0001*

[1]ANOVA test, *significant, the analysis of variance showed that there was a significant (*P*=0.0001) difference in CFU among the groups

Table 2: Comparison of CFU between Group 2 and Group 3

Groups	CFU/ml (Mean±SD)
Group 2	151.87±32.58
Group 3	28.40±12.50
P-value[1]	0.0001*

[1]*Post hoc* tests, *significant

Table 3: Comparison of CFU between Group 1 and Group 3

Groups	CFU/ml (Mean±SD)
Group 1	315.13±63.97
Group 3	28.40±12.50
P-value[1]	0.0001*

[1]*Post hoc* tests, *significant

Table 4: Comparison of the number of CFU/ml between Group 1, Group 2, and Group 3

Groups	CFU/ml (Mean±SD)
Group 1	315.13±63.97
Group 2	151.87±32.58
Group 3	28.40±12.50
P-value[1]	0.0001*

[1]ANOVA test, *significant

Seltzer *et al*. demonstrated that even instrumentation short of the apex would set up an inflammatory response in the periapical area. Ingle and Beveridge state that files are likely to force bacteria from the apical third of the canal into the surrounding tissue and referred to this as the "worm" of necrotic debris.[6] It was recommended that irrigation should be used to clean the canal. Grossman believed that the root canal instruments act as a plunger, forcing material ahead of it, and through the apical foramen. Many factors affect the amount of extruded debris such as the improper use of irrigating solutions, long canals, instrumentation methods, canal diameter, larger files (size 50 and above), and type of file used.[2] Irrigation or chemical debridement is accepted as being a necessary aid in the chemomechanical cleansing of root canals. Irrigation assists in debris removal. More debris is removed when greater quantities of irrigating solutions are use.[2] Furthermore, the proximity of the irrigation needle to the apex plays an important role removing root canal debris.[4] The effectiveness of irrigation is questioned in narrow canals, with smaller needles being suggested for better exchange of solution and cleaning.[5]

In contrast, the push-pull filing action of the crown-down technique tends to push more debris apically. Furthermore, the crown-down instrumentation produced significantly more debris with bacteria than rotary nickel–titanium techniques. This is in agreement to the studies conducted by Mckendry, Al-Omari, and Dummer.[8] The present study also gives result in accordance to above statement that manual crown-down technique extruded more debris apically.

In the present study, the chief objective was to evaluate the apical extrusion of bacteria induced by rotary file systems. This study evaluated two of such rotary file systems along with crown-down hand filling system which is widely used and used in continuous rotation. They are

1. Group 1 – 21 mm No. 15-40 K Files (Mani Inc., Tochigi, Japan)
2. Group 2 – ProTaper next (Dentsply Maillefer, Switzerland)
3. Group 3 – One Shape (Micro-Mega, Besancon, Cedex).

Evaluation of rotary multiple file technique in a study by Roane *et al*. that utilize ProTaper next files resulted in less extrusion of debris apically.[7] They tend to pull dentinal debris into the flutes of the file and direct it toward the coronal aspect of the canal. These are in agreement to the studies conducted by Visse and Brilliant.[9] In the present study, ProTaper next rotary files showed less extrusion of debris apically as compared to crown-down hand filling technique. Recently, many advances have been taking place in the preparation techniques and instruments employed. The crown-down or cervical flaring type preparation results in less apical extrusion of debris by reducing the amount of dentine available to push apically and creating a space large enough for debris to be rinsed away in a coronal direction.[5] In the present study, the rotary instrumentation utilizes the crown-down technique and hence results in less extrusion of debris apically. ProTaper next extruded a significantly higher amount of bacteria, as compared to One Shape system. The ProTaper system is a faster system with less number of instruments. Elmsallati *et al*. reported that the file with short pitch design extruded less debris them medium and long ones. ProTaper is a long pitch design instrument while One Shape is a short pitch instrument.[10]

The increased taper is believed to be more efficient at enlarging root canals, in a "crown-down" manner and negotiating and preparing curved root canals with predictable results.[11] The present study was conducted with manual hand file, rotary ProTaper next (Dentsply Tulsa Dental Specialty), and One Shape single file system (Micro-Mega, Besancon, France).

The ProTaper next files used in this study were Ni–Ti rotary instruments. It has ISO standardized tip with Taper 2%. It uses triangular blank, which provides more flexibility when compared to square blank. It uses more flexible stainless steel. Flutes are more tightly twisted to give more cutting edges.[12]

The One Shape file used in this study was stainless steel instruments; angle of the tip is reduced so the file stays centered in the original canal and cuts all sides more evenly. Non-cutting tips of ProTaper next files ensure less gouging of external wall and reduced cavity transport.[9] The tip of the file has been modified to have a compound angle of 70° and 35° without active cutting edge. The purpose of such a design is to guide the tip through curvature and to reduce the risk of ledging and perforation. Reddy and Hicks were the first to compare the apical debris extrusion between hand instrumentation and engine-driven nickel–titanium instruments.[13] The instruments are made by grinding three equally spaced.

Conclusion

The following conclusions have been drawn from this study:

1. The conventional crown-down technique with linear filing motion results in significant extrusion of irrigant and debris (bacteria)
2. The rotary technique with rotatory motion results in lesser extrusion of irrigant and debris (bacteria)
3. The best way to minimize the extrusion of debris or bacteria is by adapting crown-down technique. Use of engine-driven single file instrumentation techniques significantly reduces the apical extrusion of irrigant and debris (bacteria). This study was conducted to compare the extrusion of bacteria which extruded apically from extracted teeth after endodontic instrumentation using the manual hand and rotary instrumentation techniques. The study samples comprised 45 intact freshly extracted human premolars which were selected according to the following criteria:
   1. Free of caries
   2. Free of restorations
   3. With straight roots, the 45 extracted teeth selected based on the above criteria were thenrandomly

divided into three groups comprising 15 teeth each. A conventional access cavity was prepared in each tooth and patency of canal was determined with size 15 K-file. The canals of each group were prepared by one of the following techniques using the specific instruments:

- In Group-I: Root canals were instrumented with manual hand file used in simple crown-down technique.
- In Group-II: Root canals were instrumented with ProTaper next used in rotary technique.
- In Group-III: Root canals were instrumented with One Shape rotary system.

In all the three groups, irrigation was performed with 1 ml distilled water after the use of each instrument. Each tooth was assessed for the volume of irrigants and debris or bacteria extruded apically. The teeth were placed in a vial. The apical part of the root was suspended within the vial, which acted as a collecting container for irrigants and apical debris or bacteria evacuated through the foramen of the root.

The bacteria count was carried out on an electronic colony counter apparatus and the values were statistically analyzed. The results showed that hand instrumentation technique showed the greatest extrusion of both irrigant and debris ($P < 0.05$).

Rotary technique extruded significantly smaller amounts of irrigant and debris compared to manual hand filing technique ($P < 0.05$).

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